POMEGRANATE AND GARDEN CRESS SEED OILS AS POTENTIAL SOURCES OF OMEGA FATTY ACIDS FOR OIL BLENDS BY

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ABSTRACT

Fatty acids profile of pomegranate seed oil (PSO) and garden cress seed oil (GCSO) as well as their blends with sunflower seed oil (SFSO) at different ratios was chromatographically analyzed. The blending ratios of SFSO with PSO were 80:20, 60:40 and 40:60 (w/w), while blending ratios of SFSO with GCSO were 75:25 and 50:50 (w/w), respectively. PSO characterized by its higher content (84.16%) of punicic acid (ω–5 fatty acid) which has beneficial effect on human health. Blending of SFSO with PSO resulted in reduction of linoleic acid ($C_{18:2}$) and an increase in punicic acid with increasing of the ratio of PSO in the blend. However, blending SFSO with GCSO, the ω–3 fatty acids increased in blend samples as the ratio of GCSO increased. So, erucic acid in blends with GCSO did not exceed 5% in agreement with WHO recommendation for edible oils. Therefore, pomegranate and garden cress seed oil can be used as potential sources for improving the nutritional value with omega–3 and omega–5 acids in edible oils.

Key Words: Garden cress seed oil, Nutritional value, Omega–3 fatty acid, Pomegranate seed oil, Punicic acid, Sunflower oil.

INTRODUCTION

Pomegranate (*Punica granatum* L), member of the family Punicaceae, is one of the most ancient edible fruits. They are widely

grown in Mediterranean regions (including Iran) and India, but sparsely cultivated in the USA, China, Japan and Russia **(De Melo *et al.*, 2014)**. Total worldwide production of this crop is approximated at 1,500,000 tons and Iran with the highest area under cultivation produces 47% of world production. The edible parts of pomegranate fruits are consumed fresh and are also used in the preparation of fresh juice. The fruit contains considerable amounts of seeds, ranging between 40 and 100 g kg^{-1} of fruit weight depending on cultivar **(Dadashil *et al.*, 2013).**

Pomegranate leaves has a dark green color with a glossy shape, it has a leathery leaves that are narrow and lance–shaped. Leaves size ranges from 3-7 cm long and 2 cm broad. Blossoms produced in summer where rainfall in minimal during late summer. The pomegranate flower has a bright red color with 5-8 crumpled petals which persists on the fruit and the flower size is 3 cm in diameter **(Dipak *et al.*,2012)**.

Pomegranate has great benefits for human health. Pomegranate juice has a potential antioxidant and anti-inflammatory activities and pomegranate seed oil (PSO) has been reported to promote epidermal tissue regeneration **(De Melo *et al.*, 2014)**. Pomegranate is a food with a lot of benefits for human. Pomegranate juice (PJ) or its extracts in routine supplementation may correct or prevent obesity, diabetes and cardiovascular diseases. The pomegranate leaf extract has a free radical scavenging activity **(May and Fozia**, **2012)**.

Pomegranate seed oil (PSO) has several features that make it an attractive nutraceutical ingredient as a consequence of its novelty, good acceptance by the consumers, cheap availability and promising phytochemical composition. PSO forms or compose 10-20% of total seed weight, with an ideal highly unsaturated fatty acid content, mainly constituted by the rare conjugate linolenic acid punicic acid **(Dadashil *et al.*, 2013).** The punicic acid is the main fatty acid (FA) in the (PSO), it ranged from 73% to 79% of total FA in seed oil by comparing with its percentage in the other seed oils such as bitter ground (60%), pot marigold (29.5%) and catalpa (27.6%). As the PSO has the highest amount of conjugated linolenic acid (CLnA) than the same well-known CLnA-rich seeds, the PSO could potentially serve as a dietary source for CLnA for reducing risks of cancer and obesity **(Jing *et al.*, 2012)**.

Mahdi and Navaei (2006) mentioned that garden cress was used by ancient Egyptians as a food source and became well known in various parts in Europe, including Britain, France, Italy and Germany. In spite of this, it is still used as a minor crop.

Garden cress is an annual, herbaceous edible plant that is botanically related to mustard and watercress. This plant is native to Egypt and South West Asia. It is cultivated in India, North America and parts of Europe as it is known as garden pepper, grass, pepper cress, pepperwort or poor man's pepper. Garden cress seeds are small, oval-shaped, pointed and triangular at one end, smooth, about 3-4 mm long, 1-2 mm wide, reddish brown in color **(Doke and Guha, 2014).**

Garden cress oil showed a presence of eight major fatty acids namely: palmitic (10%), stearic (2.9%), oleic (22%), linoleic (12%), linolenic (34%), arachidic (3.4%), eicosendic (12%) and erucic (4.4%). The saturated acids content was as low as 15.6 % and the unsaturated acids content was as high as 84.4% in garden cress seed oil **(Sumangala *et al.*, 2004 and Diwakar *et al.*, 2010).**

Our study was carried out to determine the fatty acid composition for pomegranate seed oil and garden cress seed oil as well as evaluating the effect of their blending with sunflower oil at different ratios 20, 40 and 60% for pomegranate seed oil and 25 and 50% for garden cress seed oil on omega fatty acids content.

MATERIALS AND METHODS

MATERIALS:

Pomegranate (*Punica granatum* L.) fruits were obtained from the local market at Giza, Egypt, the mean weight of each fruit was 250 gm. The pomegranate seeds were directly isolated from the fruits by pressing using juice extractor and then seeds were obtained from the arials, washed and dried at room temperature (25 ± 2 ^{o}C) for 24 hrs.

Garden cress seeds ***(Lepidium sativum)*** were obtained from Harraz Spices Co. Cairo, Egypt.

Refined Bleached Deodorized sunflower oil was obtained from Arma Company, 10th of Ramadan city, Egypt.

METHODS:

Extraction of oil: each of pomegranate or garden cress seeds were dried, powdered in a plate mill. Oil extraction process was

performed by Soxhelt apparatus using petroleum ether as a solvent (b.p. 40-60°C) for 6 hrs **(AOAC 2010).** The solvent was evaporated using rotary evaporator apparatus and the extracted oil was dried over Na_2SO_4 anhydrous, filtered and stored at –18°C ± 2 until use for analysis.

Preparation of oil blends: Sunflower seed oil was blended with pomegranate seed oil at different percentages of 20, 40 and 60% (w/w), whereas, blends with garden cress seed oil were performed at ratios of 75:25 and 50:50 (SFSO:GCSO, w/w).

Fatty Acid Analysis: The fatty acid composition was released as methyl ester according to **IOOC (2001)** as following:

In 5 ml screw-top test tube approximately 0.1 g of the oil sample was weighed, 2 ml of heptanes was added and shacked well. 0.2 ml of 2N methanolic potassium hydroxide solution was then added, putting on the cap fitted with PTFE-joint, the cap was tightened and the tube was shacked vigorously for 30 seconds then left to stratify until the upper solution became clear. The upper layer was decanted that contained the methyl esters. The heptanes solution was then injected into the gas-liquid chromatography instrument (HP 6890N, Agilent Technologies, USA) equipped with a split/splitless injector (split ratio 1:100) with flame ionization detector (FID) and DB-23 capillary column of 60m × 0.32mm, ID 0.25µm film thickness was filled with 50% cyano-propyl-methyl-poly-siloxane. The injector temperature was 250°C and the detector temperature was 275°C. The temperature of the column was programmed initially at 150°C then allowed to rise at a rate of 10°C/min till reaches 170°C then allowed to rise at a rate of 5°C/min till reaches 192°C then holding at this temperature for 4 min. Then allowed to rise at a rate of 10°C/min till reached 220°C then held at this temperature for 3 min. Nitrogen gas was used as carrier gas with constant flow rate 3.0 ml/min. Hydrogen gas was used as fuel gas with constant rate 3.3 ml/min and air with constant rate 33 ml/min. The volume injected was 1 µl and injected into the column by a Hamilton micro syringe.

The fatty acids present in the sample under investigation were thus identified by comparison of relative retention times (RT) and peak areas which are estimated by computerized process, then these peak areas were used to calculate the fatty acid content expressed as percentage of total fatty acids, applying the following relationship:

$$\textbf{Area\% Fatty Acid}_X = \{A_X / A_T\} \times 100$$

Where;

A_X = area counts of fatty acid methyl esters X

A_T = total area counts for chromatogram

The standard solution composed of standard methyl esters of fatty acids used for identification and matching of samples, it contained the following methyl esters fatty acids: palmitic (16:0), palmitoleic (16:1), heptadecanoic (17:0), heptadecanoic (17:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), eicosanoic (20:1), behenic (22:0), docosanoic (22:1), punicic (18:3) and lignoceric (24:0). This standard was obtained from Sigma Chemical Company, USA.

Iodine value:

The iodine value (g I_2 / 100g oil) was calculated from the fatty acid profile according to **Kyriakidis and Kasiloulis (2000)** from the following equation:

$$\textbf{IV}_{\textbf{calculated}} = \textbf{XC}_1 + \textbf{YC}_2 + \textbf{ZC}_3$$

Where; for GCSO and PSO: X=1.00; Y=1.50; Z=2.62
for SFSO: X=0.95; Y=1.60; Z=2.62

C_1: sum of unsaturated fatty acids with one double bond

C_2: sum of unsaturated fatty acids with two double bonds

C_3: sum of unsaturated fatty acids with three double bonds

RESULTS AND DISCUSSION

The fatty acids composition of pomegranate and garden cress seed oils as well as their blends with sunflower oil were chromatographically determined and the data are presented in **Tables 1, 2 and 3**. From the obtained results, it could be observed that the major fatty acids in pomegranate seed oil were the conjugated linolenic acid (CLNA) called punicic acid $C_{18:3}$ being 84.16%, followed by oleic acid (4.75%) and linoleic acid (4.11%). These results coincide with those reported by **Topkafa *et al.* (2015) and Hennessy *et al.* (2016).** The other fatty acids were: palmitic acid (2.14%), stearic acid (1.78%), $C_{22:0}$ (1.73%) and traces of $C_{16:1}$, $C_{17:0}$, $C_{17:1}$, $C_{18:3}$ n3, $C_{20:0}$ and $C_{20:1}$ as shown in **Table (1).** The obtained results agreed with **Kyralan *et al.* (2009).**

It could be noticed that the saturated fatty acids consisted 6.11% from the total acids, however, the unsaturated acids proved the higher content being 93.88% and characterized by its highest concentration of punicic acid.

Regarding the fatty acids profile for garden cress oil, it could be noticed from data given in **Table (1),** that the major fatty acids in garden cress oil were linolenic acid $C_{18:3}$ n3 (31.29%), oleic acid $C_{18:1}$ (20.53%), eicosanoic acid $C_{20:1}$ (13.04%), linoleic acid $C_{18:2}$ (11.04%) and palmitic acid $C_{16:0}$ (8.27%). The fatty acid composition of garden cress oil under investigation was in agreement with data observed by **Sumangala *et al.* (2004) and Youssef *et al.* (2014).**

Garden cress seed oil analysis indicated that the most abundant saturated fatty acid was palmitic acid, whereas the main unsaturated fatty acids present are linolenic, oleic, eicosanoic and linoleic acids. The total saturated acids content was as low as 18.65% and the total unsaturated acids content was as high as 80.39%. These obtained results agree with **White (2007)** who reported that garden cress seed oil contained 15.6% of saturated fatty acids, whereas, the unsaturated acids were 84.4%.

Considering erucic acid content in garden cress seed oil, it was 5.79%. This obtained result agree with **Diwakar *et al.* (2010).**

On the other hand, the major fatty acids in sunflower seed oil were linoleic acid $C_{18:2}$ (59.32%), oleic acid $C_{18:1}$ (27.62%), palmitic acid $C_{16:0}$ (7.13%) and stearic acid $C_{18:0}$ (3.44%). These results agree with those reported by **Grunvad *et al.* (2013).** It could be noticed from data given in **Table (1)** that sunflower oil was free from conjugated linolenic acid (CLNA). Also, saturated fatty acids in sunflower seed oil were higher (11.67%) than that of pomegranate seed oil (6.11%) and lower than that of garden cress seed oil (16.65%).

When blending sunflower seed oil (SFSO) with different levels of pomegranate seed oil (PSO), it could be generally observed that, increasing the blend ratios of PSO reduced $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ acids depending on the addition level of PSO to sunflower seed oil. For example, oleic acid in SFSO decreased from 27.62% to 24.04, 19.59 and 14.83% when PSO was added at 20, 40 and 60%, respectively. The same trend was observed for linoleic and palmitic acids. On the other hand, addition of PSO to SFSO resulted in the presence of punicic acid in SFSO reaching 12.91, 25.60 and 45.93%

when addition levels were 20, 40 and 60% of PSO, respectively as shown in **Table (2).** So, supplementing SFSO with PSO improved the omega acids status and nutritional value of SFSO.

As for blending sunflower seed oil with garden cress seed oil (GCSO), the addition ratios were 25:75 and 50:50 for GCSO:SFSO. It could be noticed that, the addition of GCSO to SFSO decreased the oleic acid $C_{18:1}$ of SFSO from 27.62% to 25.66% when GCSO was added at level of 25:75, also linoleic acid $C_{18:2}$ decreased from 59.32% to 48.51% at the same level of addition. The rate of reduction in these fatty acids was increased with increasing the addition level of GCSO to SFSO as shown in **Table (3).**

On the other hand, the addition of garden cress seed oil improved the linolenic acid $C_{18:3}$ (n3) content as high as 7.44% and 13.96% when it was added at the ratios of 25:75 and 50:50 (GCSO: SFSO), respectively.

As for erucic acid $C_{22:1}$ it was decreased from 5.79% in GCSO to 1.11% and 2.14% in blended SFSO samples at the investigated ratios of 25:75 and 50:50 (GCSO:SFSO), respectively. These obtained results are conforming to the WHO norms for erucic acid content in edible oils.

The iodine values of pomegranate, garden cress and sunflower seeds oils as well as their blends at different ratios were calculated from the chromatographically analysis of their fatty acids profile. It could be noticed that the iodine value of pomegranate seed oil revealed higher value being 232.89 than other oils samples due to its high content of unsaturated fatty acids especially the punicic acid.

However, garden cress seed oil (GCSO) showed iodine value of 149.02, while the iodine value of sunflower seed oil was calculated to be 118.69g I_2/100gm oil. The obtained data reflected the highest level of unsaturated fatty acids in such oils. These results agreed with those reported by **Habibnia *et al.* (2012) and Diwaker *et al.* (2010).**

The blends of pomegranate seed oil with sunflower oil at ratios of 20:80, 40:60 and 80:20 (w/w) showed an increment trend in iodine values being 136.64, 149.02 and 180.36, respectively. This observation indicates the increasing trend of the unsaturated fatty acids in sunflower oil as result of blending by pomegranate oil. Also, the same observation was noticed for sunflower oil samples blended with

garden cress seed oil at the ratios of 25:75 and 50:50 (w/w) being 122.68 and 126.54gm I_2 /100gm oil, respectively.

It could be stated that the blend levels of garden cress seed oil at 25 and 50% were chosen to reduce the erucic acid, whereas, supplementing sunflower seed oil up to 50% of garden cress seed oil is the best level which gave erucic acid content less than 5%, at the same time improved the nutritional value of sunflower oil.

From the above obtained results, it could be mentioned that pomegranate seed oil and garden cress seed oil can be considered as a potential, alternate and non–conventional seed oil for omega–3 and omega–5 (punicic acid) and can be blended with suitable edible oils or in the form of a supplement to increase omega fatty acids in functional foods.

Table (1). Relative percentage of fatty acid profile of garden cress seed oil, pomegranate seed oil and sunflower seed oil

Fatty acid	**GCSO***	**PSO***	**SFSO***
$C_{14:0}$	–	–	0.11
$C_{16:0}$	8.27	2.14	7.13
$C_{16:1}$	0.19	0.02	0.12
$C_{17:0}$	0.04	0.04	0.08
$C_{17:1}$	0.07	0.003	0.34
$C_{18:0}$	3.09	1.78	3.44
$C_{18:1}$	20.53	4.75	**27.62**
$C_{18:2}$	11.04	4.11	**59.32**
$C_{18:3}$ n6	0.14	–	0.19
$C_{18:3}$ n3	**31.29**	0.05	0.35
$C_{20:0}$	4.01	0.42	0.34
$C_{20:1}$	13.44	0.79	0.22
CLNA	–	**84.16**	–
$C_{22:0}$	2.14	1.73	0.68
$C_{22:1}$	5.79	–	–
$C_{24:0}$	–	–	0.13
Σ SFA**	17.51	6.11	11.91
Σ USFA**	82.39	93.88	88.15
Iodine value	136.23	232.89	118.69

* GCSO: Garden cress seed oil, PSO: Pomegranate seed oil, SFSO: Sunflower seed oil
**SFA: Saturated Fatty Acids, USFA: Unsaturated Fatty Acids

Table (2). Relative percentage of fatty acid profile of pomegranate seed oil and sunflower seed oil blends at different levels

Fatty acid	20 %PSO* + 80% SFSO*	40%PSO* + 60%SFSO*	60%PSO* + 40%SFSO*
$C_{14:0}$	0.04	0.04	0.03
$C_{16:0}$	6.78	5.69	4.71
$C_{16:1}$	0.11	0.08	0.06
$C_{17:0}$	0.05	0.05	0.05
$C_{17:1}$	0.02	0.02	0.01
$C_{18:0}$	3.03	2.78	2.48
$C_{18:1}$	24.04	19.59	14.83
$C_{18:2}$	51.48	40.71	29.28
$C_{18:3}$ n6	0.15	0.91	0.08
$C_{18:3}$ n3	0.28	0.22	0.17
$C_{20:0}$	0.34	0.36	0.38
$C_{20:1}$	0.44	0.42	0.55
CLNA punicic	**12.91**	**25.60**	**45.93**
$C_{22:0}$	0.30	3.53	1.43
$C_{22:1}$	-	-	-
$C_{24:0}$	-	-	-
Σ SFA**	10.54	12.45	9.08
Σ USFA**	89.43	87.55	90.91
Iodine value	136.64	149.02	180.36

* PSO: Pomegranate seed oil, SFSO: Sunflower seed oil
**SFA: Saturated Fatty Acids, USFA: Unsaturated Fatty Acids

Table (3). Relative percentage of fatty acid profile of garden cress seed oil and sunflower seed oil blends at different levels

Fatty acid	25 %GCSO* + 75 % SFSO*	50% GCSO* + 50 % SFSO*
$C_{14:0}$	0.07	0.98
$C_{16:0}$	8.25	8.49
$C_{16:1}$	0.16	0.16
$C_{17:0}$	0.05	0.05
$C_{17:1}$	0.04	0.03
$C_{18:0}$	3.16	3.14
$C_{18:1}$	25.66	24.29
$C_{18:2}$	48.51	38.30
$C_{18:3}$ n6	0.24	0.21
$C_{18:3}$ n3	7.44	13.96
$C_{20:0}$	1.08	1.79
$C_{20:1}$	2.86	5.35
CLNA	–	–
$C_{22:0}$	0.63	0.72
$C_{22:1}$	1.11	2.14
$C_{24:0}$	0.74	0.38
Σ SFA**	13.98	15.55
Σ USFA**	86.02	84.44
Iodine value	122.68	126.54

* GCSO: Garden cress seed oil, SFSO: Sunflower seed oil
**SFA: Saturated Fatty Acids, USFA: Unsaturated Fatty Acids

CONCLUSION

In this study it could be concluded that edible oils can be blended with pomegranate or garden cress seed oils, up to 60% for blending with pomegranate oil and 50% for garden cress oil. These non–traditional seeds can be considered to be a potential alternate and non–conventional seed oil sources for omega–3 and omega–5 fatty acids and can be blended with suitable edible oils or in the form of a supplement to increase omega fatty acids in functional foods.

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زيت الرمان وحب الرشاد كمصدر للأحماض الدهنية الأوميجا لمخاليط الزيوت

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** قسم الزيوت و الدهون – معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – الجيزة

الملخص العربي

تم تحليل تركيب الأحماض الدهنيه لكل من زيوت الرمان وحب الرشاد ودوار الشمس وكذا خليط كل من زيت دوار الشمس مع زيت الرمان بنسب 80 : 20 , 60 : 40 , 40 : 60 وكذلك زيت دوار الشمس مع زيت حب الرشاد بنسب 75 : 25 , 50 : 50 وتم التركيز على الأحماض الدهنية بالتحليل الكروماتوجرافى ووجد أن زيت الرمان تميز بإرتفاع محتواه من الحمض الدهنى البانثيك (أوميجا–5) بلغت 84.16 % و الذي له فوائد صحية، بينما تميز زيت حب الرشاد بإرتفاع محتواه من الحمض الدهنى ك$_{18:3}$ (أوميجا–3) 31.29. أدت عملية خلط زيت الرمان مع زيت دوار الشمس إلى إرتفاع محتوى الأخير من الحمض الدهنى البانثيك (أوميجا–5) بينما أدت عملية خلط زيت حب الرشاد مع زيت دوار الشمس إلى إرتفاع نسبة الحمض الدهنى ك $_{18:3}$ (أوميجا–3) في زيت دوار الشمس كما لوحظ أن عملية خلط زيت حب الرشاد مع زيت دوار الشمس أدت الى خفض المحتوى من الحمض الدهنى الإيروسيك الى الحد الموصى به من توصيات منظمة الصحة العالمية (أقل من 5 %) في الزيوت الغذائية ولهذا أوضحت الدراسة أنه يمكن الاستفادة من زيوت الرمان وحب الرشاد كمصدر للأحماض الدهنية الأوميجا في الزيوت الغذائية و منتجاتها.